COMPARISON OF IMMUNOGENECITY AND SAFETY OF DPT-HB COMBINATION VACCINE AND DPT & HEPATITIS B VACCINES GIVEN SEPARATELY

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CERTIFICATE

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DECLARATION

I declare that this dissertation entitled "***Comparison of Immunogenecity and safety of DPT-HB combination vaccine and DPT & Hepatitis B vaccines given Separately*** " has been conducted by me at the Institute of Child Health and Hospital for Children. It is submitted in part of fulfillment of the award of the degree of M.D (Pediatrics) for the March 2007 examination to be held under the Tamil Nadu Dr. M.G.R Medical University, Chennai. This has not been submitted previously by me for the award of any degree or diploma from any other university.

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CONTENTS

INTRODUCTION

Immunisation of children with Diphtheria, Pertusis and Tetanus is now universal in most countries of the world and has considerably helped to diminish the morbidity and mortality from these vaccine preventable diseases.

DIPHTHERIA

Diphtheria is an acute infectious disease caused by cornynebacterium diphtheriae. It is an ancient disease which used to occur in epidemics and was associated with high mortality.

Corynebacaterium diphtheriae is an irregularly staining gram positive, non-motile, nonspore forming, pleomorphic bacillus. The pathogenicity of the organism is due to the release of exotoxin. Diphtheria toxin is lethal to man in an amount of about 230µg/kg.

Diphtheria spreads worldwide. The only known reservoir of diphtheria is human beings.

The disease affects mainly unimmunised children, below 15years of age. Even though majority of the cases are still reported from developing countries, over the years its incidence has gone down considerably due to immunization.

PERTUSIS:

Pertusis is an acute, highly communicable infection of the respiratory tract caused by Bordetella pertusis. It may affect any susceptible host but is common and serious in infancy and early childhood. It should be considered with high index of suspicion in an unimmunised child presenting with severe spasmodic cough. In India before the introduction of EPI, there were more than 2.5 lacs of cases reported in 1974 and 4.5 lacs in 1978[1]

Mortality in pertusis was around 6 percent [2] . Similar high incidence has been reported in 1950's in developed countries, but after the introduction of vaccine, incidence of pertusis came down significantly. In India during postvaccination era, there has been a decline in incidence of pertusis.

Epidemics of pertusis are reported every 3 to 4 years in UK. Most recent estimates of WHO indicate that 12.9 million die of ARI[3]. Pertusis accounts for 0.26 million of them (6.1 percent of ARI death). It is thus clear, that even with decline in pertusis after vaccination, it still continues to be a major health hazard. Infants may be susceptible before the age at which vaccine is immunogenic, postvaccinal immunity is likely to wane off in older children who may suffer from mild or modified form of disease that is unrecognizable.

Of course, unimmunised children in the community continue to suffer and spread infection.

TETANUS:

Tetanus is an acute illness caused by a soluble exotoxin of the bacterium clostridium tetani. The name "***Tetanus***" means arching of the body due to stiffening, which is a prominent feature of this disease.

Tetanus occurs worldwide and is an important cause of neonatal deaths in developing countries. The causative organism clostridium tetani is part of the normal flora in human and animal intestine and is disseminated through the excreta.

The contamination of the wounds, unhygienic and improper handling of the umbilical cord in newborns, lack of hygienic habits and aseptic care during and after delivery in women are the chief risk factors in infection.

According to a WHO report (1992) 112 countries reported a total of 19,882 cases of tetanus of which 15, 394 cases or neonatal tetanus. Of these, India reported 5915 cases of neonatal tetanus[4]. The incidence is high in tropical countries with humid climate. More cases are reported from rural than urban areas.

Tetanus usually manifests in a generalized from, but sometimes it may be local, as seen in children with otits media. Localised tetanus produces pain and spasm of the muscles in proximity of the site of injury. The fatality rate of the generalized form of disease is around 10 percent [5]

Tetanus neonatorum is usually a serious and often fatal disease. It usually begins at the age of 3 to 10 days.

DPT VACCINE:

For immunizing infants, the preparation of choice is DPT. Firstly because, the infant can be immunized simultaneously against three disease viz., Diphtheria, pertusis and tetanus, which is a great gain administratively. Secondly the pertusis component in DPT vaccine enhances the potency of the diphtheria toxoid.

There are two type of DPT vaccine plain and adsorbed. Adsorption is usually carried out on a mineral carrier like aluminum phosphate or hydroxide. Studies have shown that adsorption increases the immunological effectiveness of the vaccine. The WHO recommends that only adjuvant DPT preparation be utilized in immunization programmes [6.]

The composition of DPT vaccines in India is shown in table:

Contents	***Glaxo***	***Kasuli***
Diphtheria toxoid	25Lf	30Lf
Tetanus toxoid	5Lf	10Lf
Pertusis (millions)	20,000	32,000
Aluminium phosphate	2.5mg	3mg
Thiomersal B.P.	0.01%	0.01%

The plain (DPT) vaccine can be used as a booster.

STORAGE:

DPT vaccines should not be frozen. They should be stored in a refrigerator between +2 to +8 C. The vaccine should be used before the date of expiry indicated on the vial. When issued to a sub-centre, the vaccine should be used within a week. The vaccine will lose potency if kept at room temperature over a longer period of time.

OPTIMUM AGE:

It has been found that young infants respond well to immunization with potent vaccines and toxoids even in the presence of low to moderate levels of maternal antibodies. According, the Global advisory group of the EPI, has

recommended that DPT vaccine can be safely and effectively administered, as early as 6 weeks after birth [7].

NUMBER OF DOSES:

Three doses of DPT, is considered optimal for primary immunization. Furthermore, in almost all recipients, three doses of DPT are associated with higher and more sustained levels of Diphtheria and tetanus antitoxin and acceptable level of pertusis protection.

INTERVAL BETWEEN DOSES:

The current recommendation is to allow an interval of 4 weeks between the 3 doses with a booster injection at one and half years, followed by another booster (DT only) at the age of 5 years. Studies have shown that two months intervals do not offer any advantage over one month interval for protection against diphtheria and tetanus and may not enhance pertusis protection.

MODE OF ADMINISTRATION:

All vaccines containing mineral carrier or adjuvants should be injected deep intramuscular. This applies to DPT also, which may be given in the anterolateral aspect of the thigh.

REACTIONS:

Fever and mild local reactions following DPT immunization are common. It is estimated that 2 to 6% of vaccinese develop fever of 39 C or higher and that 5 to 10% experience swelling and indurations or pain lasting more than 48 hours [8]

The most severe complications following DPT immunization are neurological and are thought to be due primarily to the pertusis components of the vaccine, the estimated risk is 1:170,00 doses administered [9]

CONTRAINDICATIONS:

DPT should not be repeated if a severe reaction occurred after a previous dose. Such reactions include.

1. Collapse or shock like state
2. Persistent screaming episodes
3. Temperature above 40 c
4. Convulsions and other neurological symptoms.
5. Anaphylactic reactions.

Since the severity of pertusis infection decreases with age, the pertusis component in DPT vaccine is not usually recommended after the age of 5 years.

Therefore children over the age of 5 years who have not received DPT, need only 2 doses of DT vaccine 4 weeks apart with a booster dose 6 months to 1 year later.

Those children who received the primary course of DPT earlier, should receive only DT as a booster at 5 years of age.

For immunizing children over 12 years of age and adults, the preparation of choice of DT, which is an adult type of diphtheria-tetanus vaccine. This preparation contains no more than 2 Lf of diphtheria toxoid per dose, compared with 25 Lf in the ordinary (Pediatric) DPT Vaccines.

Administration of DT vaccine to adults is carried out in 2 doses at an interval of 4 to 6 weeks followed by a booster 6 to 12 months after the second dose.

HEPATITIS-B

Ever since the identification of the structure of the Hepatitis-B (HBV) and the noble prize winning work of characterizing the Hepatitis B surface (HBsAg), efforts have been on to develop a safe effective vaccine against the Hepatitis B virus. Through intensive effects of microbiologists, epidemiologists and clinicians, we are now at the stage where such a vaccine is available. Hence it is possible to interrupt transmission of this infection,

resulting in tremendous reduction in morbidity and mortality from the disease in the long term.

Hepatitis B infection has a usual incubation period ranging from 1-6 months, following which the infected person suffers from loss of appetite, nausea, vomiting, abdominal pain and Jaundice.

Some individuals remain asymptomatic during the acute infection, very rarely an infected individual many die of fulamianant hepatitis during the acute stage, within days or weeks. More commonly, the initial symptomatic or asymptomatic infection subsides; the individual recovers and develops lifelong immunity. However, some persons develop chronic infection.

The age of initial infection is a major factor determining the outcome. [10] It has been noted that fewer than 10% of children below 5 years of age are symptomatic when they first become infected but 30-50% of them develop chronic infection later in life.

The figure for chronicity goes as high as 80-90% for infants who get infected during the first year of life. In contrast, 30-50% of adults are sick when they first become infected, but only 2-5% develop chronic infection. Further 15 to 25% of chronically infected persons are likely to die of hepatocellular carcinoma or cirrhosis in later life. Thus, chronic infection and

its sequelea are mostly responsible for the morbidity and mortality caused by Hepatitis B.

BURDEN OF HEPATITIS B INFECTION IN INDIA:

For any clinical condition, disease burden is calculated on the basis of the morbidity and mortality caused by the disease. In the case of Hepatitis B, it may not be possible to calculate morbidity and mortality caused by acute infection, as many infected persons remain asymptomatic and hence the acute phase may be missed altogether. Further, mortality during acute infection is not unduly high, therefore it is not a good reflector of disease burden. Hence a calculation of disease burden based on these indicators is likely to be misleading. The real risk of Hepatitis B infection is the risk of long term viral carriage and replication with the risk of chronicity and it's complications including chronic liver disease, cirrhosis, hepatocellular carcinoma and death. Thus the prevalence of these clinical conditions in a population may indirectly suggest the burden of HB infection [11]

Although exact figures for chronic liver disease, cirrhosis and mortality due to Hepatitis B infection have not been calculated in India, in population studies, data generated at referral centers suggests that around 40,000 deaths annually are attributable to Hepatitis B infection. It has also been estimated that around 11,000 new cases of Hepatocellular carcinoma occur every year for

which the hepatitis B virus may be responsible in a large majority. The overall risk of mortality from various complications of chronic HB infection is about 25%. Applying this figure to the fact that about 4% of India's population carries the HBsAg, it translates to one death in every hundred being caused by Hepatitis B[12.] Using this calculation Hepatitis B has been categorized as a significant health problem in India.

Most of the 50 odd Indian studies with varying sample size among different population group have reported HBsAg prevalence around 2-4% although it ranges from as low as 0.7% as high as 10%.

Based on the prevalence of HBsAg in the population, the world is roughly divided into three zones of endemicity. India is categroised in the intermediate group.

Another newly emerging concept is infection with mutant strains which can lead to chronicity even in the absence of the surface antigen, such an infection may be detectable only by finding HBV DNA (i.e.) other markers are absent. This group is important because

1. They may not be picked up on routine screening.
2. Their numbers are reportedly increasing.

3. The currently available vaccines may not protect against mutant infection.
4. A mathematical model recently worked out suggests that owing to mutations, HBV strains will become resistant to the currently available vaccine in about 20 years.

As mentioned previously, the earlier the age of initial infection with HBV, the higher is the risk of developing chronicity. The risk is thus maximum for babies infected during birth or early infancy.

The results of the various small studies indicates that perinatal transmission may account for 29-55% of the carrier load in India.

Besides the studies that suggest a rather high incidence of perinatal transmission, there is another body of evidence that indirectly suggests this fact, which is the analysis of age specitic HBsAg prevalence. The prevalence of HBsAg positivity and anti HBsAg in preschool children is either higher or not significantly different from older children and adolescents, suggesting that most of the infection has occurred before 5 years of age. The overall prevalence of carriers in preschool children is 2 to 2.5%. Further age stratified data shows almost uniform prevalence of HBsAg and antibody from 6 months onwards, suggesting that most, if not all under 5 children have been infected in the first half of infancy itself. From this it is reasonable to conclude that, most

of this infection would have been acquired perinatally, since exposure to HBV at this age is more likely to have occurred by this route than horizontally. Thus, perinatal transmission in India is high and is possibly major route of transmission[13]

PREVENTION OF HBV INFECTION:

Since chronically infected persons are the main reservoir of HB infection, strategies for prevention are focused on this group. The WHO has suggested that three major strategies may be helpful in interrupting transmission of HBV. These include

1. Routine infant vaccination to prevent child to child transmission.
2. Interruption of perinatal transmission.
3. Vaccination of older age individuals at risk of infection.

This three pronged strategy covers the three high risk groups for HB infection viz. Newborn babies of infected mothers, children and older persons at risk.

HEPATITIS B VACCINE:

There are two types of vaccines against HB infection, which are plasma derived vaccines and recombinant vaccines.

The former are prepared by purifying plasma of persons with chronic HBV infection, and using the HBsAg therein . Following the widespread usage of the vaccine, the number of infected individuals will decease and the availability of plasma will also dwindle, making its manufacture difficult.

The second type of vaccine is a genetically engineered preparation where in plasmids containing the genetic material required for the production of the surface antigen are incorporated into the genetic material of a yeast (sacchromuyces cerevisiae) which then begin to produce the surface antigen.

The two types of vaccine did not differ significantly in their safety profile, immunogenicity and sero-efficacy. In recent years the recombinant vaccine has gained popularity and is almost universally used.

WHO SHOULD BE VACCINATED?:

It is clear that children and even newborn babies are at risk of contracting HB infection, therefore the best strategy would be to vaccinate all children including newborn babies, thereby reducing the risk of HB infection at the earliest possible age.

Selective immunization to high risk groups like those in the medical profession, professional blood donars, drug abusers, those engaged in hetero or homosexual promiscuity is unlikely to interrupt successfully the transmission of the infection.

Based on these considerations in 1991 the EPI Global Advisory group of the WHO recommended that HBV vaccine should be integrated into National Infant Immunisation programs of all countries with a carrier rate of 8% or greater by 1995 and in all remaining countries by 1997[14]

The immunization committee of IAP also recommended incorporation of Hepatitis B vaccine into universal infant immunization to the government of India several years back, although this has been accepted and begun to be implemented only in recent months.

AT WHAT AGE SHOULD VACCINATION BEGIN?

In a population where perinatal transmission is significant, unless all pregnant women are screened for HBsAg, it is impossible to predict which baby is likely to be infected, implying that proportion of newborn babies will be infected at birth itself. In such a situation, administration of vaccine would be akin to post exposure prophylaxis, which obviously must be administered as soon as possible in order to be effective. This is the rational of recommending

the first dose of vaccine at birth itself, because optimum efficacy is achieved when the vaccine is given with in the first 24 hours after birth. A delay of even seven days renders the vaccine ineffective against perinatal transmission of infection[15]

SCHEDULE OF IMMUNIZATION:

The latest recommendations of the advisory committee on immunization practices (ACIP) and the American Academy of family physicians (AAFP) also specify that the recommended interval between the first and second doses of Hepatitis B vaccine is 4 weeks. It is also recommended that there should be a gap of at least 8 weeks between second and third doses, though the interval could vary form 2 to 17 months.

However such a schedule makes the incorporation and integration of HB vaccine into existing immunization schedules a cumbersome exercise. Therefore attempts were made to delay the four weeks dose to six weeks, and the results have been acceptable. However in order to fully integrate the HB schedule into the existing schedule, the doses should be administered at one month interval, along with the DPT vaccine. Trials have shown that giving the third dose 4 weeks after the second dose results in seroconversion but with a lower GMT of antibody. But this GMT of antibody is in the protective range (0.1 Iu-1 Iu)[16]

Based on these considerations, the Indian academy of pediatrics recommended two schedules for infants.

1. The first is to vaccinate babies at birth, 6 weeks and 14 weeks. The schedule is in agreement outlined earlier and will also be effective against perinatal transmission.
2. The second option is to give the three doses of HB vaccine at the same time as DPT namely 6, 10 and 14 weeks.

Obviously, this strategy will not prevent perinatal transmission. This discrepancy has been explained as a strategy so as not to deprive babies who miss the birth dose, from getting at least some degree of protection.

It remains debatable whether a strategy that is unlikely to yield maximum benefit should be pursued at all, particularly in light of the fact that giving an option of delaying HB vaccine, may make some parents opt for the later administered schedule. This dual schedule strategy has not been evaluated in terms of protective efficacy and long term benefits.

COMBINATION VACCINES:

Infectious diseases are the world's leading cause of death and vaccines are the most effective tool to combat them. The number of vaccines is

increasing over the last 15 years. If all these vaccines are included in a active immunization schedule the number of injections to administer all vaccines are quite high.

According to children's vaccine initiative launched in 1990, also the ideal vaccine would provide all indicated antigens in a single dose and would be heat stable, effective when administered soon after birth and affordable to families of all economic levels.

But some studies have shown altered immune responses to various vaccines when they are given combined with other vaccines, similarly adverse events after combined vaccine are generally said to be increased [17]

COMBINATION VACCINE TYPES:

There are two basic types of combination vaccines:

1. In the first type, multiple different antigens of a microbial agent are mixed together as single product to confer immunization against the multiple serotypes of disease causing agent. These include vaccines against poliomyelitis and pneumococcal disease (capsular polysaccharide antigens).
2. In other type, multiple different antigens of different pathogens are mixed together to immunize against different disease such as vaccines

against Diphtheria, Pertusis and Tetanus. In the further, new design of combination vaccines,DNA Technology or use of non pathogenic vectors capable of expressing multiple vaccine antigens.

ISSUES IN COMBINATION VACCINES:

With the increasing availability and use, several important issues need to be considered to make the best possible use of these vaccines against the prevention of vaccine preventable diseases.

These are listed below:

1. There should be antigenic compatibility between different antigens.
2. The immunogenic response to each component should be adequate.
3. The product should have a shelf life for a period of 18-24 months.
4. There should be an appropriate timing for the use of each antigen in on immunization schedules.
5. The side effects should be at an acceptable level. There may be additional concerns such as difficulties in assessing the vaccine component in case of an adverse event following immunization.

BENEFITS OF COMBINATION VACCINES:

1. To the child and family:
    a. Multiple antigens are given with single injection, reducing number of injections and total number of visits.

b. There is reduced parental anxiety and pain to child leading to higher compliance, low dropout rates.

2. To the health planner:

a. Reduce storage place, packing, handling and transport.

b. Reducing burden on cold chain.

c. Reducing documentation and logistics.

ISSUES CONCERENED WITH COMBINATION VACCINES:

1. IMMUNOGENICITY

The combination of vaccines may produce decreased immunogenicity of the individual components because of physical interaction among the vaccine components. For example some studies have shown that addition of Hepatitis B antigen with DPT, decrease the immunological response to Diphtheria and Tetanus toxoid. .This may be due to cross interference of antigens in the combination vaccine [18]

The other reason may be due to the combination of one vaccine which is administered with adjuvant, with another vaccine not given with adjuvant may lead to the displacement of the adjuvant and the decreased immune response to first antigen. Additions of stabilizers, buffers and excipients may also interferes with the compatibility between the difference antigens.

2. ADVERSE EVENTS

A study done by G. Papaevangelou, et al in Greece have shown that adverse effects both local and systemic are more frequent in combination vaccine group (DPT-HB) than separately given DPT and Hepatitis B vaccine. They have reported that this may be due to the use of adjuvant, stabilizers and buffers in combination vaccine. (DPT-HB).

3. COST OF THE COMBINATION VACCINE

Cost of the combination vaccine is many a times higher than the individual formulation. This is a very important factor to be concerned in a developing country like India.

REVIEW OF LITERATURE

I. Dr. Yong Poovarawan et al conducted a study comparing the Immunogenticy and safety between DPT –HB combined vaccine and DPT and Hepatitis –B given separately.

This study was conducted in Department of Pediatrics, Chulalohgkorn University and Hospital, Bangkok, Thailand

It was a Randomised control trial with 50 subjects in combined vaccine group and 50 subjects in separate vaccine group

They assessed the Immunogencity and safety profile between the two groups.

Their conclusion was that the antibody titre for Diphtheria and Tetanus was more in the separately given vaccine when compared to combined vaccine and it was also statistically significant.

The frequency of adverse reactions also was more in the combined vaccine group when compared to separate vaccine group.

They concluded that although the combined vaccine has the advantage of having been given by single injection, the separately given (DPT & HB) vaccine is more Immunogenic and has a good safety profile.

II. A similar study was conducted by Dr. G. Papavagelou et.al in Department of Peadiatrics, West Africa Hospital, Athens, Greece.

It was also a Randomised control trial with 60 subjects in combined vaccine group and 60 subjects in separate vaccine group.

The results of their study showed that antibodies to Diphtheria was more in separate vaccine group when compared to combined vaccine group but antibody titres against Pertusis, Tetanus and Hepatitis B was more or less equal between the two groups with out any statistical significance.

But separate vaccine group got a good safety profile when compared to combined vaccine group.

III. A similar study was conducted by a pharmaceutical company in Lithuanian infants.

In that study about 150 subjects was enrolled .75 subjects Randomised to combined vaccine group and another 75 subjects Randomised to separate vaccine group.

They also studied the immunogenicity and safety profile between the two groups.

They concluded that combined vaccine (DPT-HB) is equally immunogenic and has a good safety profile comparing to separate vaccine group.

STUDY JUSTIFICATION

In our region there is no study comparing the immunogenicity and safety of DPT-HB combination vaccine and DPT & Hepatitis-B vaccines given separately. So, in this study we are going to compare the immunogenicity and safety between the combination vaccine and vaccines given separately.

AIM

The aim of this study is to ***find out*** and ***compare*** the Immunogenicity and safety of DPT-HB combination vaccine and DPT & Hepatitis B vaccines given separately.

MATERIALS AND METHODS

STUDY DESIGN	:	Randomised Control Trial.
STUDY PLACE	:	Immunisation Clinic, Institute of Child Health & Hospital for Children.
STUDY PERIOD	:	March 2005 to March 2006.
STUDY POPULATION	:	Infants at 6-8 weeks of age brought to Immunisation Clinic.
SAMPLE SIZE	:	60.
SAMPLING TECHNIQUE	:	Simple random.

INCLUSION CRITERIA

Full term, Normal healthy infants at 6-8 weeks of age.

EXCLUSION CRITERIA

Previous vaccination or infection with DPT.

Administration of Immunoglobulims, or any other blood products since birth.

Major congenital or Hereditary immuno deficiency.

Those who have not given consent.

MANEUVER

After getting consent from the parents the subjects will be allocated in each group with the use of a standard table of Randomisation. Peripheral venous blood of 2 ml is withdrawn, centrifuged and the serum will be stored in deep freezer at 20 Celsius.

The serum will be sent to the laboratory for Pre-vaccination titre evaluation.

After 3 doses of vaccination, Postvaccination sample will be withdrawn 3 to 6 weeks after the last dose. The serum will be sent to the laboratory for Postvaccination titre evaluation.

A diary card will be issued to the subject after each dose of vaccination.

The parent or guardian will be asked to note any adverse effects both local and systemic, in the Diary Card. And they will be asked to reproduce the Dairy card during the next visit.

SCHEDULE OF VISITS AND OBSERVATIONS

Visit/ Procedures	***Visit 1***	***Visit 2***	***Visit 3***	***Visit 4***
Visit Intervals	6-8 weeks after birth (1st dose)	4-6 weeks after 1st dose	4-6 weeks after 2nd dose	3-6 weeks after 3rd dose.
Written informed consent	ü	-	-	-
Eligibility Criteria	ü	-	-	-
Vaccination	ü	ü	ü	-
Blood Sampling	ü	-	-	-
Assessment of local & systemic reactions	ü	ü	ü	ü
Diary cards provided	ü	ü	ü	-

DETAILED DESCRIPTION OF STUDY VISITS

Visit –1

6- 8 weeks after birth.

General Physical examination.

Informed consent form signed by legally acceptable representative.

Blood sampling for estimation of antibodies.

First dose of vaccine.

Assessment of local and systemic adverse reactions for the initial 30 minuteso one hour at the study hospital.

Give dairy card 1 to the subject with instruction.

Visit –2

Collect dairy card ***1***.

Check for any exclusion criterion.

Second dose of vaccine.

Local and systemic adverse reaction evaluation for the initial 30 minutes to one hour at the study hospital.

Give diary card ***2*** to the subject with instruction.

Visit –3

Collect diary card 2.

Check for any exclusion criterion.

Third dose of vaccine.

Local and systemic adverse reaction evaluation for first 30 minutes to one hour.

Give diary card 3 to the subject with instruction.

Visit –4

Collect diary card ***3***.

Local and systemic adverse reaction evaluation.

General physical examination.

Blood sampling for estimation of antibodies.

IMMUNOGENICITY

Blood samples for the estimation of antibody titers will be collected before and three to six weeks after the third dose. The antibodies estimated will be Anti –Diphtheria (IgG-ELISA NOVOTEC),Anti-pertusis(Ig G ELISA NOVOTEC),Anti-tetanus(IgG ELISA NOVOTEC) and AntiHBsAg ELISA.

SAFETY

Local and systemic adverse affects will be mentioned after each dose. Close monitoring for 30 minutes to 1 hour after each dose.

Both solicited and unsolicited local and systemic reactions will be recorded on the diary card by the parent till the next dose.

All subjects will be followed up for local and systemic reactions even after 30 days after the 3rd dose of vaccine.

PREMATURE TERMINATION

Subjects will be withdrawn from the study and referred for appropriate care in the following cases,

1) Any clinical adverse event, intercurrent illness or other medical condition which indicates to the investigator that continued participation is not in the best interest of the subject.

2) The subjects will be withdrawn in case of any serious adverse events.

ADVERSE EVENT REPORTING

ADVERSE EVENTS:

Any untoward medical occurrence in a subject or clinical investigation subject during the administration of a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

LOCAL REACTIONS

A local reaction is defined as the occurrence of one or several reactions at the injection site within 28 days following vaccination. Local reactions are

- Local Pain
- Swelling
- Redness at Injection Site
- Induration
- Unsolicited local reaction.

SYSTEMIC REACTIONS

A systemic event is defined as the occurrence of one or several of the following symptoms within 28 days following vaccination. They are:

- Fever
- Persistent Cry
- Unusual Crying for more than 60 minutes

- Vomiting
- Diarrhea
- Loss of appetitie
- Restlessness

COMPOSITION

Dose of Individual Components in the combined and separate vaccines:

Active Ingredients	***Per Dose***
Diphtheria	**:** 25 Lf
Tatanus	**:** 5 Lf
Pertusis	**:** 15 U
Hepatitis B	**:** 10 micorgrams

Other Ingredients

Thiomersal	-	0.025 mg
Aluminium hydroxide	-	0.625 mg

MODE OF ADMININSTRATION

Deep Intramuscular injections in the anterolateral thigh.

DOSAGE

Three doses (0.5 ml / dose) will be administered (WHO guidelines)

Separate vaccine group:-

Visits	***Age***	***Separate Vaccine***
1	6 Weeks	DPT & HB Separately
2	10 Weeks	DPT & HB Separately
3	14 Weeks	DPT & HB Separately

Combined vaccine group:-

Visits	***Age***	***Combined Vaccine***
1	6 Weeks	DPT –HB
2	10 Weeks	DPT –HB
3	14 Weeks	DPT – HB

STORAGE CONDITION

The vaccine vials will be kept in a refrigerator at +2 to + 8 Cwith a lock.

STATISTICAL CONSIDERATION

ANALYSIS OF IMMUNOGENICITY

Analysis of Immunogencity will be evaluated by estimation of antibody titers to Diphtheria, Pertusis, tetanus and Hepatitis B.

Geometric mean titre of antibodies against Diphtheria, Pertusis, tetanus and HBsAg will be calculated then.

The recommended antibody titre for protection for each antigen according to WHO are:

Antigens	***Safe protection***	***Long term protection***
Diphtheria	0.1 to 1.0 Iu /ml	>1.0 Iu/ml
Pertusis	-	>11 NT units/ml
Tetanus	1 to 5 Iu/ml	>5 Iu/ml
HBsAg	-	>10 mIu/ml

ANALYSIS OF SAFETY

Number of subjects experiencing local and systemic reactions during follow up period.

Number of serious adverse events occurring during the follow up period.

Number and percentage of subjects with at least one local, systemic and any adverse event after vaccination and during the 4 weeks follow up period.

Number and percentage of subjects with atleast one serious adverse event, with the frequencies of each type of event.

OBSERVATION

THE FOLLOWING TABLE SHOWS THE PRE-VACINATION ANTIBODY TITRE IN THE COMBINED VACCINE GROUP:

Subject Initial	Subject ID	Diphtheria Iu/ml	Pertusis NT Units/ml	Tetanus IU/ml	Anti HBsAg mIU /ml
BOU	01	.05	5	5	3
BON	02	.05	4	3.16	2
KA1	03	.05	1	2.28	2.5
RAH	04	.04	2	1.52	1.46
THA	05	.35	2.5	1.7	1.5
ARU	06	0.24	3.6	0.26	2.6
SAT	07	0.95	9.03	3.34	107
DIN	08	.01	5.1	4.5	1.76
MOH	09	.02	4.5	2.58	4.82
CHA	110	.04	3.6	1.8	1.05
BOJ	11	.03	3.8	1.9	2.05
HAR	12	.01	4.8	1.46	3.07
VIJ	13	.05	51.1	1.46	4.5
DEV	14	0.3	1.2	1.42	1.07
MAD	15	.06	2.4	2.88	1.76
SAL	16	.04	117	1.76	1.81
BOV	17	.03	4	3.74	2.8
SIV	18	.02	3	2.7	1.56
KAN	19	.05	1	2.24	1.4
BOA	20	.01	117	.05	1.6

Continued…

PRE	21	.05	0.4	4.5	2.6
BOL	22	.04	4.5	3.48	2.1
YUV	23	.025	3.6	1.74	3.45
REN	24	.035	3.1	1.7	4.4
NIV	26	0.28	3.2	1.11	9.72
YOG	27	0.28	3.2	1.98	2.5
BOP	29	.012	51.1	2.2	.01
SHY	30	.45	4.5	2.29	1.5

1)Diphtheria

0.1 to 1.0 Iu/ml ***:*** Safe Protection

>1.0 Iu/ml ***:*** Long term protection

2)Pertusis

<9 NT Units/ml ***:*** Negative

9 to 11 NT Units/ml ***:*** Borderline

> 11 NT Units /ml***:*** Positive

3)Tetanus

0.1 to 1.0 Iu/ml ***:*** Safe Protection

>1 Iu/ml ***:*** Long term Protection

4) Anti HBsAg

< 10 mIu / ml ***:*** No Protection

>10 mIu /ml ***:*** Safe Protection.

GEOMETRIC MEAN TITRE OF ANTIBODIES IN PRE VACCINATION SUBJECTS IN THE COMBINED VACCINE GROUP:

Diphtheria	***:***	.07 Iu /ml
Pertusis	***:***	6.3 NT units /ml
Tetanus	***:***	1.8 Iu/ml
Anti H Bs Ag	***:***	5.2 mIU/ml

INTERPRETATION

The geometric mean antibody titre for Diphtheria and tetanus are already in the protective range but the antibodies to pertusis and Hepatitis B are not in the protective range.

THE FOLLOWING TABLE SHOWS THE PRE-VACCINATION ANTIBODY TITRE IN THE SEPARATE VACCINE GROUP:

Subject Initial	*Subject ID*	*Diphtheria Iu/ml*	*Pertusis NT Units/ml*	*Tetanus IU/ml*	*AntiHBsAg mIU /ml*
BOA	01	.02	17.5	1.7	9.71
VAN	02	.39	7.82	3.22	15.3
BON	03	0.71	16.9	0.5	6.44
SHA	04	.01	18.3	0.62	4.51
LOG	05	.04	15.4	2.3	2.5
KOW	06	.01	11.7	1.62	2.1
HAR	07	.04	25.2	4.72	3.0
BOK	08	00	11.5	1.84	4.24
SEN	09	.05	18	2.73	3.4
GEE	10	.43	21.4	1.84	9.71
BOM	11	.01	15.8	2.73	3.4
ABU	12	0.71	11.7	2.2	2.8
PRA	13	0.39	16.9	0.62	8.8
KAV	15	.02	7.82	0.3	4.56
ALA	16	.04	17.5	1.84	87.3
SRA	17	0.05	18	3.22	6.44
BMB	18	.04	11.7	1.7	3.4
MAM	19	.39	21.4	0.62	9.80
RAJ	20	.02	18	0.5	8.70

Continued…

BOR	21	.01	18	2.73	2.5
SHO	22	.04	11.5	1.84	2.6
SAB	23	.05	25.2	4.72	20.4
BOS	24	.04	11.7	2.3	4.51
DIV	25	.01	15.4	1.62	6.44
MAH	26	0.71	18.3	2.2	4.24
DIN	27	0.39	16.9	1.52	15.3
THA	28	.02	7.82	.62	87.3
ASW	29	.02	16.7	0.3	9.84
CHA	30	.04	17.5	0.5	9.71

1)Diphtheria

0.1 to 1.0 Iu/ml **:** Safe Protection

>1.0 Iu/ml **:** Long term protection

2) Pertusis

<9 NT Units /ml **:** Negative

9 to 11 NT Units /ml **:** Borderline

> 11 NT Units/ml **:** Positive

3)Tetanus

0.1 to 1.0 Iu/ml **:** Safe Protection

>1 Iu/ml **:** Long term Protection

4) Anti HBsAg

< 10 mIu / ml **:** No Protection

>10 mIu /ml **:** Safe Protection

GEOMETRIC MEAN TITRE OF ANTIBODIES IN PREVACCINATION SUBJECTS IN THE SEPARATE VACCINE GROUP:-

Diphtheria	-	.04 Iu/ml
Pertusis	-	9 NT units / ml
Tetanus	-	1.5 Iu/ml
Anti HBsAg	-	7.5 Iu/ml

Interpretation

The geometric mean antibody titre to Diphtheria and Tetanus are in the protective range but the antibodies to pertusis and Hepartitis B are not in the protective range.

THE FOLLOWING TABLE SHOWS THE POSTVACCINATION ANTIBODY TITRE IN THE COMBINED VACCINE GROUP:

Subject Initial	*Subject ID*	*Diphtheria Iu/ml*	*Pertusis NT Units/ml*	*Tetanus IU/ml*	*Anti HBsAg mIU /ml*
BOU	01	.08	56.3	3.1	813
BON	02	.15	46.3	1.64	854
KAI	03	1.88	58.5	2.16	1285
RAH	04	1.01	28.5	.23	198
THA	05	,64	64.9	7.38	34.2
ARU	06	.53	46	.68	93.8
SAT	07	1.10	30.4	1.64	975
DIN	08	.48	35.7	.34	1004
MOH	09	.87	50.1	1.44	1094
CHA	10	.57	60.5	2.36	495
BOJ	11	10.5	49.9	4.26	36.6
HAR	12	0.52	52.9	2.43	750
VIJ	13	<.05	35.9	3.25	690
DEV	14	.31	36.9	2.42	875
MAD	15	.82	60.6	1.52	883
SAL	16	.64	54.5	1.76	180
BOV	17	.54	55.1	1.24	598
SIV	18	1.04	42.7	6.50	168
KAN	19	.39	43.5	0.27	422
BOA	20	.85	31.5	1.72	1846

Continued…

PRE	21	0.59	36.2	1.3	31.4
BOL	22	0.20	64.4	1.7	393
YUV	23	0.44	33.8	1.64	48.8
REN	24	0.25	51.9	1.46	1710
THL	25	<05	59.7	0.39	737
YOG	27	0.54	62	1.98	203
VIN	28	0.53	64.9	0.54	532
BOP	29	.09	66.1	1.98	692
SHY	30	.24	27.9	1.84	195

1)Diphtheria

0.1 to 1.0 Iu/ml **:** Safe Protection

>1.0 Iu/ml **:** Long term protection

2) Pertusis

<9 NT Units /ml **:** Negative

9 to 11 NT Units /ml **:** Borderline

> 11 NT Units/ml **:** Positive

3)Tetanus

0.1 to 1.0 Iu/ml **:** Safe Protection

>1 Iu/ml **:** Long term Protection

4) Anti HBsAg

< 10 mIu / ml **:** No Protection

>10 mIu /ml **:** Safe Protection

GEOMETRIC MEAN TITRE OF ANTIBODIES IN POSTVACCINATION SUBJECTS IN THE COMBINED VACCINE GROUP:-

Diphtheria	***:***	***0.4 /Iu ml***
Pertusis	***:***	***46.7 NT units /ml***
Tetanus	***:***	***1.5 Iu/ml***
Anti H BsAg	***:***	***403. 4 Iu/ml***

Interpretation

The geometric mean antibody titre to all the antigens in the combined vaccine group after 3 doses of vaccination are in the protective range according to WHO criteria.

THE FOLLOWING TABLE SHOWS THE POST VACCINATION ANTIBODY TITRE IN THE SEPARATE VACCINE GROUP:-

Subject Initial	*Subject ID*	*Diphtheria Iu/ml*	*Pertusis NT Units /ml*	*Tetanus IU/ml*	*Anti HBsAg mIU /ml*
BOA	01	0.74	51.8	1.52	244
VAN	02	0.52	68	4.04	257
BON	03	1.74	83	2.22	65.8
SHA	04	.86	126.3	7.14	219
LOG	05	3.76	66.4	1.62	577
KOW	06	1.16	93.2	2.16	60.4
HAR	07	0.54	46.3	1.42	100
BOK	08	1.15	19.2	2.9	411
SEN	09	.96	31.8	1.93	637
GEE	10	1.69	38.7	1.36	557
BOM	11	.41	35.7	1.36	557
ABU	12	1.67	60	4.23	369
PRA	13	.23	45.7	1.52	396
PRS	14	.84	34.5	2.24	1424
KAV	15	1.64	54	2.24	57.1
ALA	16	.55	30.3	1.9	869
SRA	17	.21	28.	2.02	1251
BMB	18	0.3	63.8	1.68	216
MAM	19	1,87	10.2	4.88	***4.16***
RAJ	20	1.04	41.1	2.7	52.2

Continued…

BOR	21	1.8	49.8	8,54	496
SHO	22	.39	9.75	2.59	728
SAB	23	.67	64.2	1.6	267
BOS	24	,63	67.4	7.84	138
DIV	25	0.4	43.8	2.44	677
MAH	26	1.27	67	1.3	197
DIN	27	.49	44.6	2.36	683
THA	28	.26	38	1.36	78.4
ASW	29	.28	35	1.46	77.2
CHA	30	1.1	81.8	2.36	241

Only one subject in the separate vaccine group is not seroconverted for Hepatitis B surface antigen.

1) Diphtheria

0.1 to 1.0 Iu/ml ***:*** Safe Protection

>1.0 Iu/ml ***:*** Long term protection

2) Pertusis

<9 NT Units /ml ***:*** Negative

9 to 11 NT Units /ml ***:*** Borderline

> 11 NT Units/ml ***:*** Positive

3)Tetanus

0.1 to 1.0 Iu/ml ***:*** Safe Protection

>1 Iu/ml ***:*** Long term Protection

4) Anti HBsAg

< 10 mIu / ml ***:*** No Protection

>10 mIu /ml ***:*** Safe Protection

GEOMETRIC MEAN TITRE OF ANTIBODIES IN POST VACCINATION SUBJECTS IN THE SEPARATE VACCINE GROUPS:-

Diphtheria	:	0.8 Iu/m
Pertusis	:	44.6 NT units /ml
Tetanus	:	2.4 IU/ml
AntiHBsAg	:	221.3 Iu/ml

INTERPRETATION

The geometric mean antibody titre to all the antigens in separate vaccine group after 3 doses of vaccination are in the protective range according to WHO criteria.

COMPARISON OF GEOMETRIC MEAN TITRE OF ANTIBODIES BETWEEN COMBINED AND SEPARATE VACCINE GROUPS IN POST VACCINATION SUBJECTS.

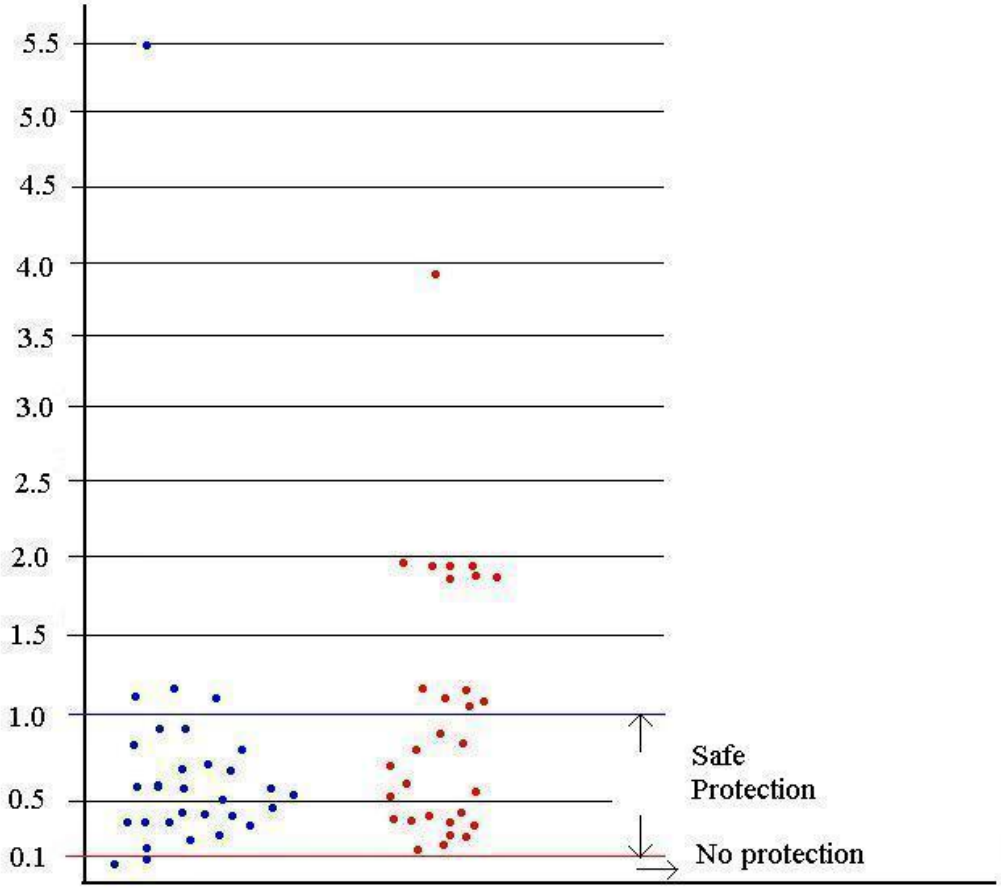
Antibodies	***Combined Vaccine***	***Separate vaccine***	***Statistical Significance (P Value)***
Diphtheria	0.4	0.8	.02
Pertusis	46.7	44.6	0.81
Tetanus	1.5	2.4	0.04
Anti HBsAg	403.4	221.3	0.05

(P<05:- Statistically Significant)

Though the antibody titres against Diphtheria, Pertusis, Tetanus and Hepatitis –B of both combined and separate vaccine group are in the protective range, the Geometric mean titre of antibodies for Diphtheria and Tetanus is better in separate vaccine group when compared to combined vaccine group and it is also statistically significant.

The antibody titre for Hepatitis B antigen in the combined vaccine group is better when compared to separate vaccine group but it is not significant statistically.

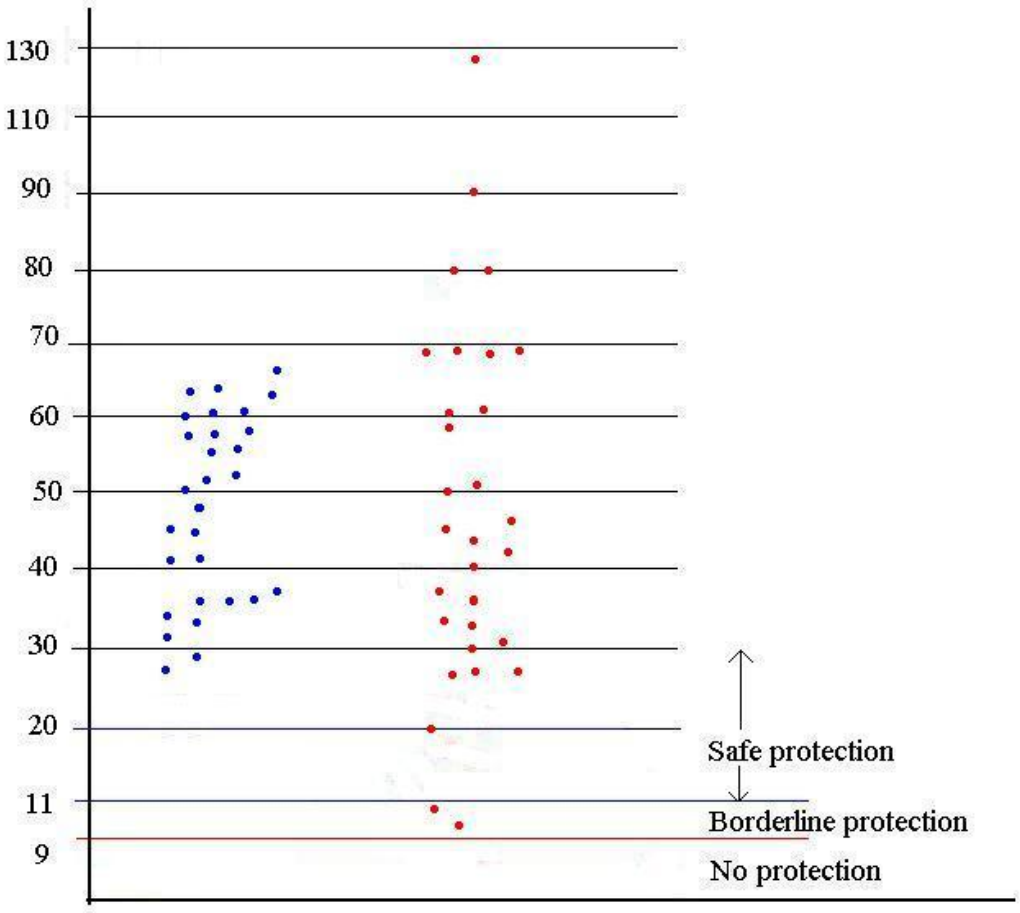
SCATOPLOT COMPARING THE POST VACCINATION ANTIBODY TITRE FOR DIPHTHERIA BETWEEN COMBINED & SEPARATE VACCINE GROUP:-



•—Antibody titre for Diphtheria in combined vaccine group

•—Antibody titre for Diphtheria in separate vaccine group

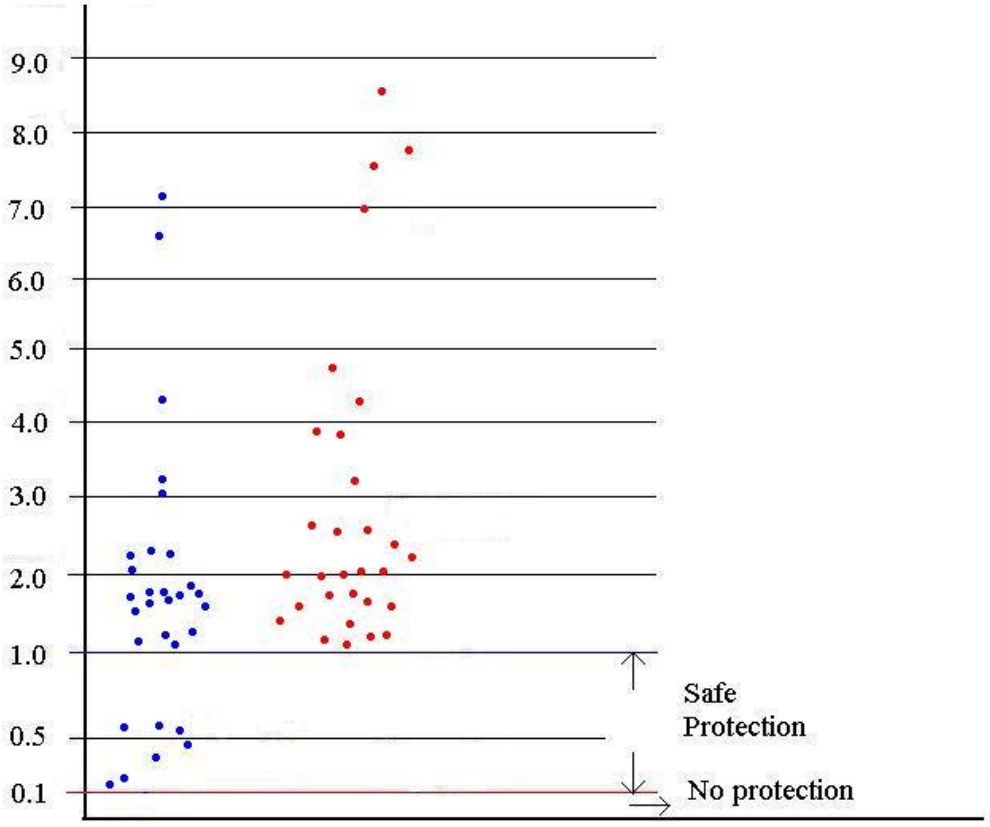
SCATOPLOT COMPARING THE POST VACCINATION ANTIBODY TITRE FOR PERTUSIS BETWEEN COMBINED & SEPARATE VACCINE GROUP:-



• — Antibody titre for pertusis in combined vaccine group

• — Antibody titre for pertusis in separate vaccine group

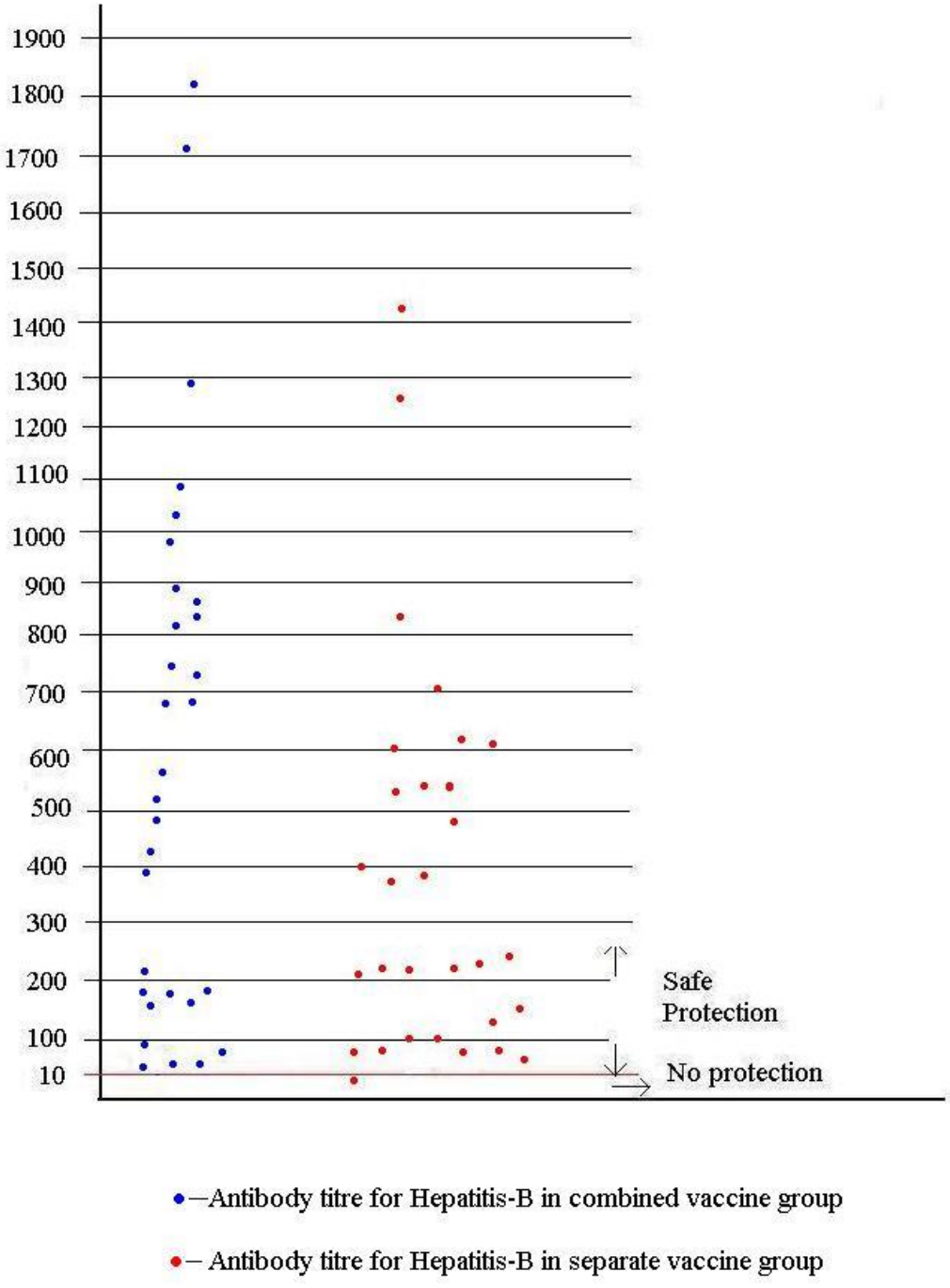
SCATOPLOT COMPARING THE POST VACCINATION ANTIBODY TITRE FOR TETANUS BETWEEN COMBINED & SEPARATE VACCINE GROUP



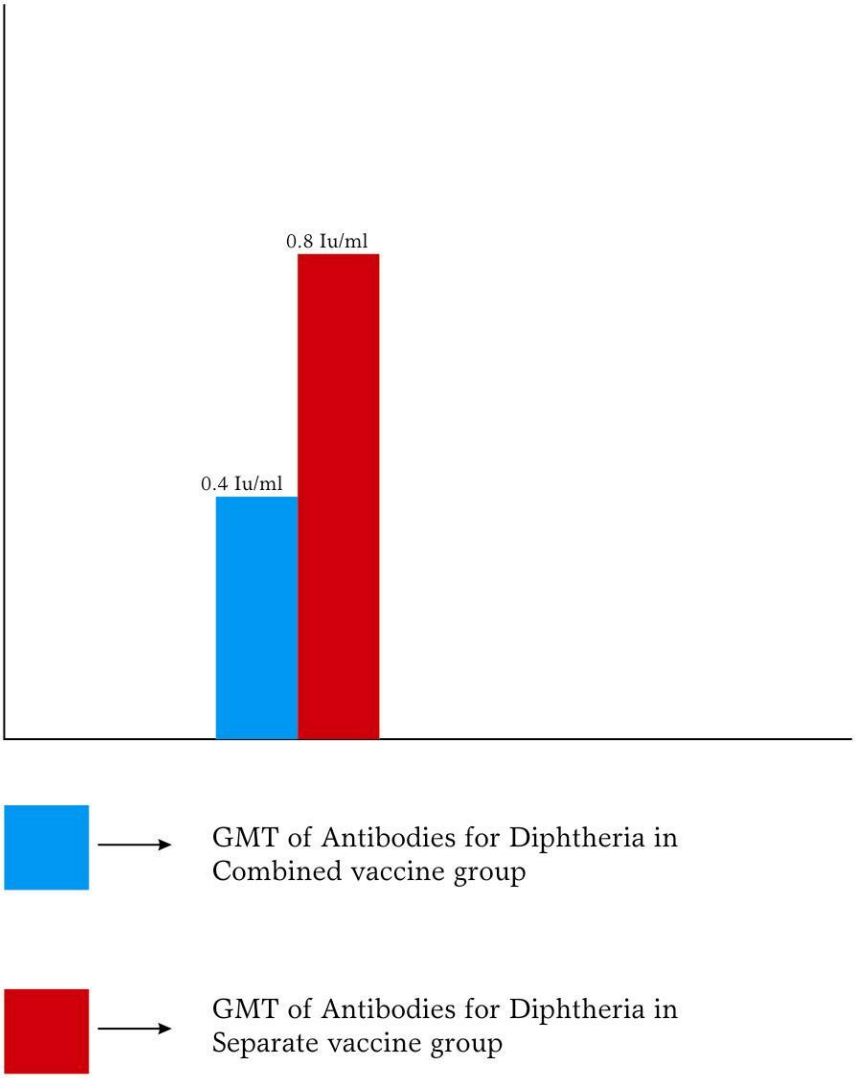
• — Antibody titre for Tetanus in combined vaccine group

• — Antibody titre for Tetanus in separate vaccine group

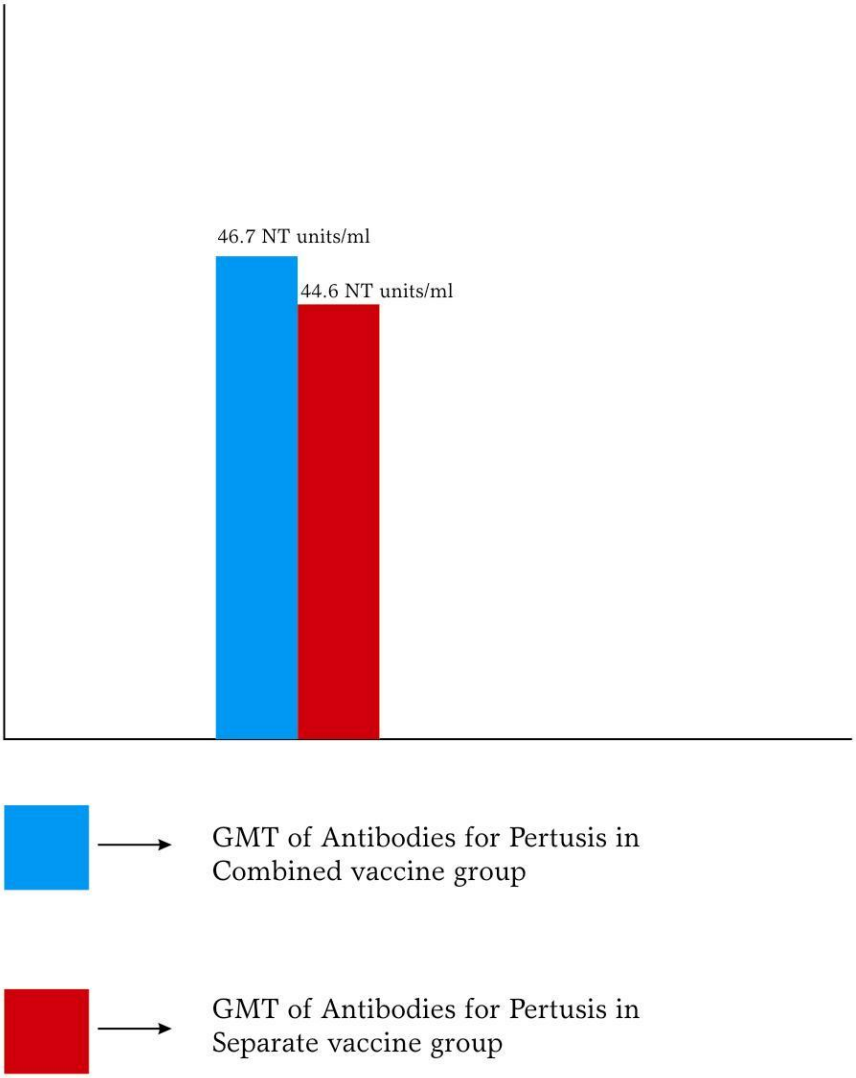
SCATOPLOT COMPARING THE POST VACCINATION ANTIBODY TITRE FOR HEPATITIS-B BETWEEN COMBINED & SEPARATE VACCINE GROUP:-



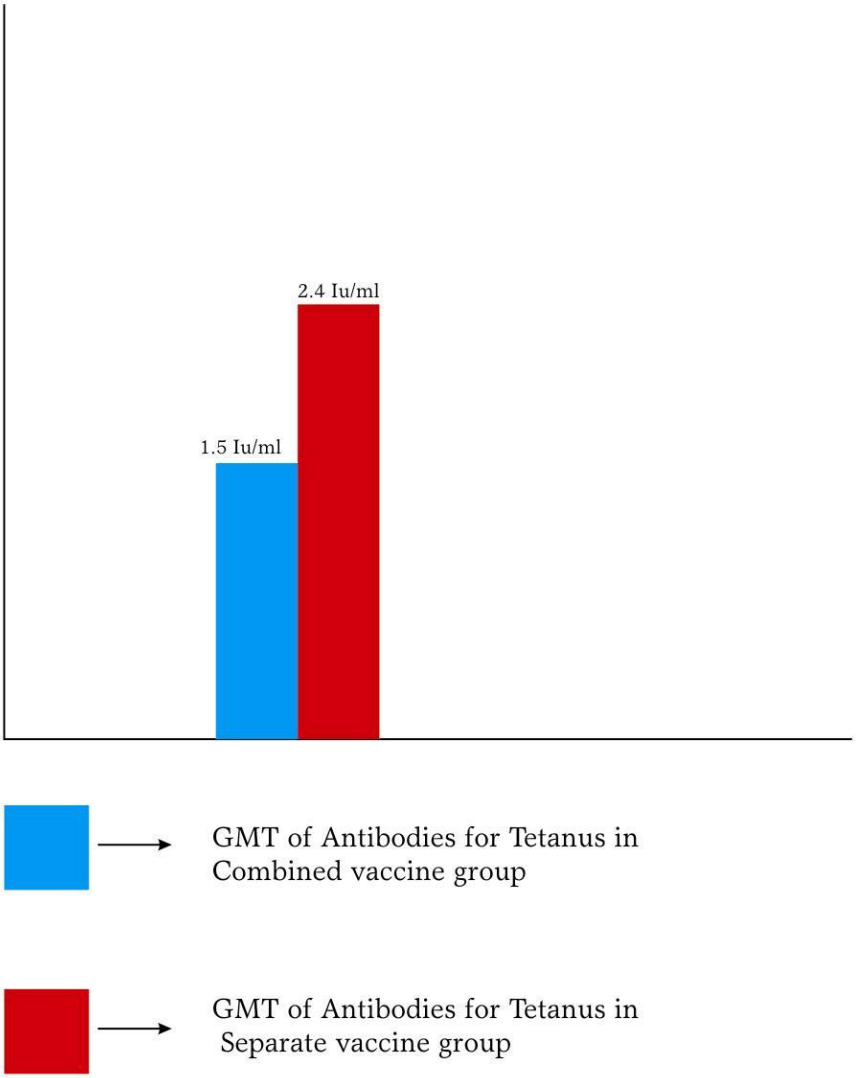
Bar Chart Comparing PostVaccnation GMT of Antibodies for Diphtheria between Combined & Separate Vaccine Group :-



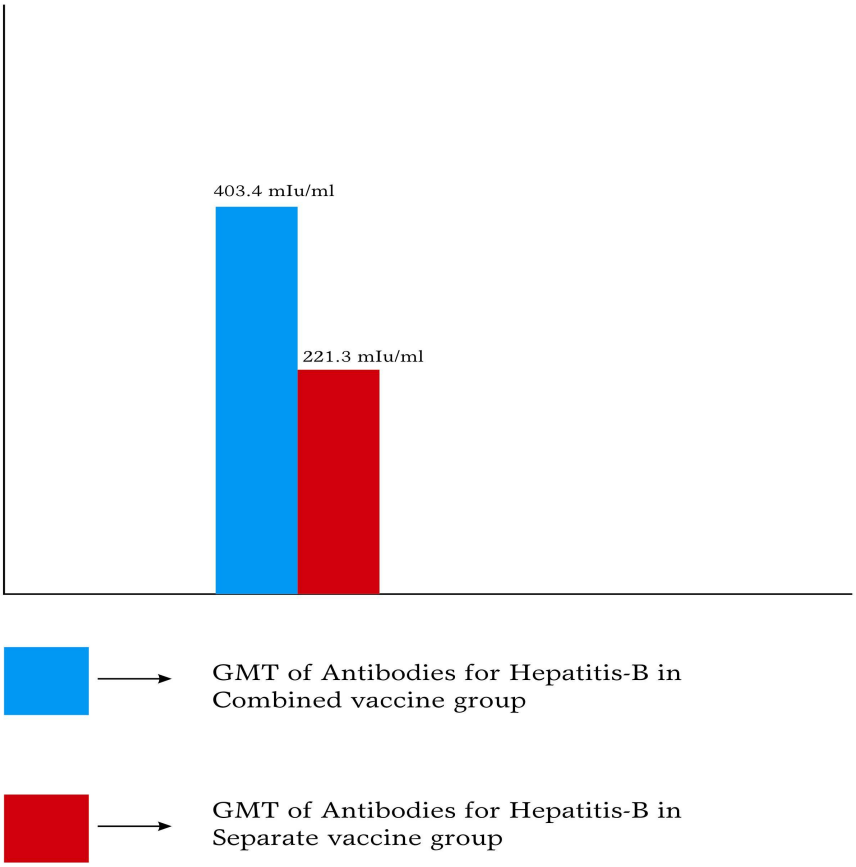
Bar Chart Comparing PostVaccnation GMT of Antibodies for Pertusis between Combined & Separate Vaccine Group :-



Bar Chart Comparing PostVaccnation GMT of Antibodies for Tetanus between Combined & Separate Vaccine Group :-



Bar Chart Comparing PostVaccnation GMT of Antibodies for Hepatitis-B between Combined & Separate Vaccine Group :-



ADVERSE EFFECTS

Local Swelling, Redness, Fever, Unusual Cry & Irritability are the commonest adverse effects noticed in both combined and separate vaccine groups.

VISIT –1

Adverse effects	***Combined vaccine (30)***	***separate vaccine (30)***	***statistical significance***
Local redness	22	15	0.06
Local Swelling	10	6	0.24
Fever	8	4	0.20
Unsual Crying	5	3	0.45
Irritability	1	0	0.31

Though adverse effects (local & systemic) are more common in the combined vaccine group when compared to separate vaccine group there is no statistical significance between them.

VISIT – 2

Adverse effects	***Combined vaccine (30)***	***Separate vaccine (30)***	***Statistical significance***
Local redness	18	12	0.05
Local Swelling	12	7	0.17
Fever	9	6	0.17
Unsual Crying	4	2	0.39
Irritability	-	-	-

There is no statistical significance in the adverse effects between the combined and separate vaccine groups after the second dose of vaccination.

VISIT-3

ADVERSE EFFECTS	***COMBINED VACCINE (30)***	***SEPARATE VACCINE (30)***	***STATISTICAL SIGNFICANE***
Local redness	16	12	0.30
Local Swelling	11	8	0.41
Fever	6	4	0.49
Unsual Crying	5	2	0.23
Irritability	2	2	0.55

Similarly ,after third dose of vaccination, the frequency of adverse effects are more in the combined vaccine group when compared to separate vaccine group but statistically it is not significant.

CONCLUSION

The frequency of adverse effects is more in the combined vaccine group when compared to separate vaccine group, but it is not significant statistically.

The Antibody titre for Diphtheria, Pertusis, Tetanus and Hepatitis B of both combined and separate vaccine groups are in the ***protective range***.

The Immunogenicity for Diphtheria and Tetanus toxoid in the separate vaccine group is better when compared to combined vaccine group and it is also statistically significant.

The clinical relevance and long term protection of these subjects needs further evaluation.

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